



**University of  
Zurich<sup>UZH</sup>**

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2010

---

## **Genetic diversity of the Pm3 powdery mildew resistance alleles in wheat gene bank accessions as assessed by molecular markers**

Bhullar, N K ; Mackay, M ; Keller, B

**Abstract:** Genetic resources of crop plants are essential for crop breeding. They are conserved in gene banks in form of a large numbers of accessions. These accessions harbor allelic variants of agronomically important genes and molecular tools allow a rapid assessment of this allelic diversity. Here, we have screened a collection of 1005 wheat gene bank accessions for powdery mildew resistance and a molecular characterization for functional alleles at the wheat powdery mildew resistance locus Pm3 was carried out mostly on the resistant accessions. The two analyzed sets of accessions consisted of 733 accessions originating from 20 different countries and 272 landraces originating specifically from Afghanistan. The Pm3 haplotype (indicating the presence of a Pm3-type of gene, susceptible or resistant) was found to be abundantly present in both sets. The accessions with a Pm3 haplotype were further screened for the presence of the functional Pm3a to Pm3g alleles using allele-specific molecular markers. Pm3b and Pm3c were the most frequently found alleles while the other five alleles were detected only in few accessions (Pm3d, Pm3e, Pm3f) or not detected at all (Pm3a, Pm3g). The data further showed that Pm3b is the major source of Pm3-mediated powdery mildew resistance in wheat accessions from Afghanistan. Susceptible allelic variants of Pm3 were found to be widespread in the wheat gene pool. The presented molecular analysis of Pm3 alleles in a diverse set of wheat accessions indicates that several alleles have defined geographical origins. Possibly, the widespread Pm3b and Pm3c alleles evolved relatively early in wheat cultivation, allowing their subsequent diffusion into a broad set of wheat lines.

DOI: <https://doi.org/10.3390/d2050768>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-43464>

Journal Article

Originally published at:

Bhullar, N K; Mackay, M; Keller, B (2010). Genetic diversity of the Pm3 powdery mildew resistance alleles in wheat gene bank accessions as assessed by molecular markers. *Diversity*, 2(5):768-786.

DOI: <https://doi.org/10.3390/d2050768>

Article

# Genetic Diversity of the *Pm3* Powdery Mildew Resistance Alleles in Wheat Gene Bank Accessions as Assessed by Molecular Markers

Navreet K. Bhullar <sup>1,†</sup>, Michael Mackay <sup>2</sup> and Beat Keller <sup>1,\*</sup>

<sup>1</sup> Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, Zurich 8008, Switzerland; E-Mail: bhullarn@ethz.ch

<sup>2</sup> Bioversity International, 00057 Maccarese, Rome, Italy; E-Mail: M.Mackay@cgiar.org

<sup>†</sup> Present address: Institute for Plant, Animal and Agroecosystem Sciences, Swiss Federal Institute of Technology, Universitätsstrasse 2, Zurich 8092, Switzerland.

\* Author to whom correspondence should be addressed; E-Mail: bkeller@botinst.uzh.ch; Tel.: +41-446-348-230; Fax: +41-446-348-204.

Received: 26 March 2010; in revised form: 22 April 2010 / Accepted: 17 May 2010 /

Published: 19 May 2010

---

**Abstract:** Genetic resources of crop plants are essential for crop breeding. They are conserved in gene banks in form of a large numbers of accessions. These accessions harbor allelic variants of agronomically important genes and molecular tools allow a rapid assessment of this allelic diversity. Here, we have screened a collection of 1005 wheat gene bank accessions for powdery mildew resistance and a molecular characterization for functional alleles at the wheat powdery mildew resistance locus *Pm3* was carried out mostly on the resistant accessions. The two analyzed sets of accessions consisted of 733 accessions originating from 20 different countries and 272 landraces originating specifically from Afghanistan. The *Pm3* haplotype (indicating the presence of a *Pm3*-type of gene, susceptible or resistant) was found to be abundantly present in both sets. The accessions with a *Pm3* haplotype were further screened for the presence of the functional *Pm3a* to *Pm3g* alleles using allele-specific molecular markers. *Pm3b* and *Pm3c* were the most frequently found alleles while the other five alleles were detected only in few accessions (*Pm3d*, *Pm3e*, *Pm3f*) or not detected at all (*Pm3a*, *Pm3g*). The data further showed that *Pm3b* is the major source of *Pm3*-mediated powdery mildew resistance in wheat accessions from Afghanistan. Susceptible allelic variants of *Pm3* were found to be

widespread in the wheat gene pool. The presented molecular analysis of *Pm3* alleles in a diverse set of wheat accessions indicates that several alleles have defined geographical origins. Possibly, the widespread *Pm3b* and *Pm3c* alleles evolved relatively early in wheat cultivation, allowing their subsequent diffusion into a broad set of wheat lines.

**Keywords:** *Pm3* alleles; powdery mildew; genetic diversity; gene banks

---

## 1. Introduction

Genetic variation forms the basis for crop improvement through breeding. Genetic diversity of different crop species is well conserved in the form of wild relatives, landraces and early varieties in the gene banks worldwide [1,2]. Plant breeding benefits from this diversity through identification of accessions that carry agronomically important genes and could potentially serve as parental accessions to develop new varieties. The use of molecular markers for evaluation of germplasm diversity among the gene bank accessions now represents an attractive alternative to the conventional phenotypic screens [3,4]. However, it is a challenging task to develop molecular markers diagnostic for a trait in wheat due to its hexaploid and large genome. Despite these difficulties, the development and use of molecular markers in wheat has strongly increased. Microsatellites (SSR markers) have been widely used to characterize genetic diversity in wheat accessions [5-8]. With the recent success in cloning of some agronomically important wheat genes, it is now possible to detect the presence of their allelic forms in a large number of germplasm accessions [9]. Among the important cloned wheat genes are the ones controlling protein content (*Gpc-B1*) [10], flowering time (*VRN1*, *VRN2*) [11,12], a domestication trait (*Q* gene) [13] and disease resistance genes (*Lr21*, *Lr10*, *Lr1*, *Lr34* and *Pm3*) [9,14-21].

Powdery mildew is one of the devastating wheat diseases and is caused by the biotrophic fungus *Blumeria graminis* f.sp. *tritici*. The identification of natural sources of resistance and breeding for resistant varieties is the most effective way to control this disease [22], as chemical control is expensive. To date, more than 37 *Pm* resistance genes have been characterized [23,24], while only one of these genes, *Pm3*, has been cloned [18]. *Pm3* is localized on the short arm of wheat chromosome 1A [25] and is now known to occur in 15 functional allelic forms (*Pm3a* to *Pm3g*, *Pm3k* to *Pm3r*). The *Pm3* alleles confer race-specific resistance to different subsets of wheat powdery mildew races [18,19,21]. *Pm3a* to *Pm3g* are the seven *Pm3* alleles that were known and characterized by classical genetic methods before the cloning of this locus [18]. The initial cloning of the *Pm3b* allele allowed the isolation of all the other *Pm3* alleles (*Pm3a* and *Pm3c* to *Pm3g*), based on the high sequence conservation between the different *Pm3* alleles. On the basis of this conservation, *Pm3* haplotype-specific markers were developed [18,21]. These markers are diagnostic for the presence of a *Pm3*-type of gene (can be a resistant or susceptible allele), although they do not identify the particular allele. Additionally, functional markers for specific detection of *Pm3* alleles *Pm3a* to *Pm3g* were developed [26]. These markers were based on nucleotide polymorphisms of the coding and adjacent non-coding regions of the *Pm3* gene and were reported to be highly diagnostic for specific *Pm3* resistance alleles. These markers were validated on different varieties and breeding lines [26]. The *Pm3*-haplotype markers and the *Pm3*-allele specific markers are very helpful to effectively screen

large sets of accessions for the presence of *Pm3* alleles. Furthermore, the long range PCR based approaches using molecular markers located in conserved regions between different *Pm3* alleles have recently allowed isolation of eight additional functional alleles of *Pm3* (*Pm3k* to *Pm3r*) from wheat gene bank accessions [9,20].

Here, we have studied the *Pm3* allele distribution in a large set of 1005 wheat gene bank accessions which originated from 20 different countries. We have used the *Pm3*-haplotype marker and the *Pm3* allele-specific markers (*Pm3a* to *Pm3g*) to determine the presence of specific *Pm3* alleles. This revealed the widespread existence of mostly susceptible *Pm3* alleles in the wheat gene pool, whereas *Pm3b* and *Pm3c* were the most abundant resistance alleles. The results also indicated *Pm3b* to be the dominant source of wheat powdery mildew resistance in landraces from Afghanistan. The relatively low frequency of the *Pm3a*, *Pm3d*, *Pm3e*, *Pm3f* and *Pm3g* alleles in gene bank accessions supports the hypothesis of a recent evolution of these alleles in the hexaploid wheat gene pool.

## 2. Experimental Section

### 2.1. Plant Material

A total of 1005 accessions were screened in this study. They were divided into two sets. The first set of 733 accessions was obtained from the gene bank of IPK, Gatersleben, Germany. These accessions originated from 20 different countries, *i.e.*, Argentina, Australia, Azerbaijan, Canada, China, Ethiopia, France, India, Iraq, Japan, Kazakhstan, Kyrgyzstan, Mexico, Nepal, Russia, Sudan, Switzerland, USA, Tajikistan and Uzbekistan. The second set of 272 bread wheat landraces from Afghanistan was obtained from the Australian Winter Cereals Collections, Australia.

### 2.2. Phenotypic Screening of the Wheat Accessions for Powdery Mildew Resistance

Detached leaf segments from seven day old plants were placed on phytagar media and were subjected to infection with powdery mildew isolates [27]. The scoring was done 9–10 days after infection. The phenotypes were classified into three categories based on the percentage of infected area on leaves: resistant (R), intermediate [(I) with two further categories: Intermediate resistant (IR) and Intermediate susceptible (IS)] and susceptible lines (S).

### 2.3. *Pm3* haplotype specific STS marker

The primer pair UP3B (5'TGGTTGCACAGACAATCC3') and UP1A (5'GAAACCCGGCATAAGGAG3') located in the *Pm3* promoter region, 4,360 bp upstream from the *Pm3* ATG start codon [18,19] was used to determine the presence or absence of the *Pm3* haplotype, indicating if a *Pm3*-type of gene is present.

### 2.4. *Pm3* Allele Specific PCRs

Allele specific PCR for *Pm3a* to *Pm3g* was carried out as described by Tommasini *et al.* [26]. The PCR conditions included an initial denaturation step at 94 °C for 3 min followed by 30 cycles of 94 °C for 45 seconds, an annealing step at variable annealing temperatures (as recommended for different primer pairs for specific *Pm3* alleles) for 35 seconds, an elongation step of 1 min per kb length of the

amplified fragment at 72 °C; and a final extension step at 72 °C for 5 min [26]. Amplification products were detected by standard gel electrophoresis on 1–1.2% agarose gels.

### 2.5. Isolation of the Full-Length Coding Sequence of *Pm3* and Sequencing

*Pm3CS* and *Pm3Go/Jho* were amplified by using *Pm3* locus-specific, long-range PCR amplification followed by a nested, long range PCR [18,21]. PCR primers were based on the upstream and downstream sequence of the coding region of the *Pm3b* allele [18]. PCR amplification of the *Pm3* alleles was carried out with the Herculase-II fusion high-fidelity DNA polymerase. Amplified fragments were cloned into the multiple cloning site of expression vector pGY1 [28]. DNA sequencing was performed with an Applied Biosystems Capillary Sequencer model 3730.

## 3. Results and Discussion

### 3.1. Detection of *Pm3* Alleles in Gene Bank Accessions Using Allele-Specific Markers

In order to determine the presence and frequency of *Pm3* alleles in wheat gene bank accessions, we first analyzed the set of 733 accessions obtained from IPK, Gatersleben Germany. All accessions were first phenotyped for powdery mildew resistance (Bhullar and Keller, unpublished data). The 154 resistant or intermediately resistant accessions were screened both for the presence of the *Pm3* gene using *Pm3* haplotype-specific markers and for the seven *Pm3* resistance alleles *Pm3a* to *Pm3g*. The *Pm3* haplotype-specific STS marker amplifies a 946bp fragment originating from the 5' non-coding region of *Pm3* [27]. It was found that 109 accessions possessed the *Pm3* haplotype and these accessions were further subjected to screening with allele-specific primers for *Pm3a* to *Pm3g*. In these 109 accessions, *Pm3c* was the most frequently detected allele, found in 17 accessions (Table 1) originating from Nepal (8), India (7), China (1) and Australia (1). In an earlier study that determined the presence of *Pm3* alleles in landraces [27], the *Pm3c* allele had been found in three landraces from Iran and one from Azerbaijan (Table 1). Although *Pm3c* was first identified in cultivar Sonora from Mexico [29,30], the data obtained here indicate that this allele has evolved in Nepal, India or close geographic areas.

The *Pm3b* allele was the second most frequent allele in the screened set (Figure 1). It was found in 6 accessions with two of these accessions originating from Russia while the remaining four were from France, Kazakhstan, Uzbekistan and Tajikistan (one each). In a previous study, *Pm3b* had been reported in 15 landraces from Afghanistan, 6 landraces each from Russia and Iran, 2 landraces from Azerbaijan and 1 landrace from Turkey (Table 1) [27]. As evident from these data, *Pm3b* was mostly detected in accessions that originated from the countries neighboring Uzbekistan, the country of its first identification in landrace Chul [30,31]. The large number of landraces from Afghanistan with *Pm3b* indicates an origin of this particular allele in this geographic region (see also 3.4).

We detected the presence of *Pm3d*, *Pm3e* and *Pm3f* alleles in 1, 2 and 2 accessions, respectively, in the screened set. The *Pm3d* allele was found in an accession from Argentina while the two accessions carrying *Pm3e* originated from India. The two accessions carrying *Pm3f* were from Argentina and China. The *Pm3a* and *Pm3g* alleles were not detected in this set. The alleles *Pm3d*, *Pm3e* and *Pm3f* had first been described in accessions originating from Afghanistan (Hindukush), Australia and USA,

respectively [32]. Thus, in contrast to *Pm3b*, we found the alleles *Pm3c*, *Pm3d*, *Pm3e* and *Pm3f* in accessions with different and distant origins compared to the places of their first identification. It is likely that the cultivars used for first identification were derived from landraces with geographical origins near the evolutionary origin of the alleles.

**Table 1.** Detection of *Pm3* alleles in gene bank accessions by allele-specific molecular markers for *Pm3a* to *Pm3g*. Accession names and countries of origin are listed.

<i>Pm3</i> allele	Number of accessions carrying the tested <i>Pm3</i> allele	Country of origin	Accession (s) in which the particular <i>Pm3</i> allele was detected	Source of the accessions (gene bank)	Reference
<i>Pm3b</i>	1	France	TRI980	IPK <sup>1</sup>	This work
	1	Kazakhstan	TRI7321	IPK	This work
	1	Uzbekistan	TRI17549	IPK	This work
	1	Tajikistan	TRI17561	IPK	This work
	2	Russia	TRI18263; TRI18742	IPK	This work
	6	Russia	VIR23918, VIR23922, VIR34986, VIR35021, VIR35030, VIR34984	VIR <sup>2</sup>	[27]
	15	Afghanistan	AUS9943, AUS9948, AUS10003, AUS10033, AUS13239, AUS13297, AUS13306, AUS13307, AUS13311, AUS14504, AUS14532, AUS14840, VIR45538, VIR49005, VIR49006	AWCC <sup>3</sup> , VIR	[27]
	6	Iran	IG122348, IG122354, IG122361, IG122373, IG122502, VIR38613	ICARDA <sup>4</sup>	[27]
	2	Azerbaijan	VIR16766, VIR31595	VIR	[27]
	1	Turkey	VIR35203	VIR	[27]
<i>Pm3c</i>	8	Nepal	TRI2437; TRI2439; TRI2448; TRI2748; TRI2765; TRI3255; TRI4029; TRI4091	IPK	This work
	7	India	TRI2799; TRI2804; TRI3375; TRI3542; TRI3552; TRI3986; TRI9986	IPK	This work
	1	China	TRI4088	IPK	This work
	1	Australia	TRI8320	IPK	This work
	3	Iran	IG122491, IG122372, IG122346	ICARDA	[27]
	1	Azerbaijan	VIR46301	VIR	[27]
	1	Argentina	TRI11472	IPK	This work
<i>Pm3d</i>	1	France	Oid HD4-266	INRA <sup>5</sup>	[19]
	2	India	TRI2554; TRI2782	IPK	This work
<i>Pm3e</i>	1	Tajikistan	TA10381	KSU <sup>6</sup>	[19]
	1	France	Oid 91-35	INRA	[19]
	1	Argentina	TRI7521	IPK	This work
<i>Pm3f</i>	1	China	TRI16947	IPK	This work
	1	France	Oid HD4-219	INRA	[19]

1 IPK: Leibniz Institute of Plant Genetics and Crop Plant Research, Germany.

2 VIR: N.I. Vavilov Research Institute of Plant Industry, Russia.

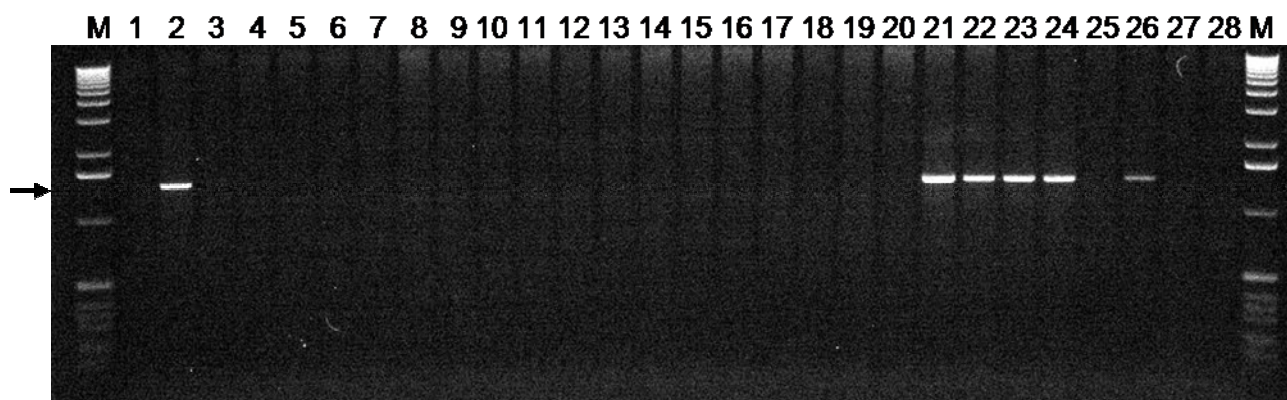
3 AWCC: Australian Winter Cereals Collection, Australia.

4 ICARDA: International Centre for Agricultural Research in the Dry Areas, Syria.

5 INRA: French National Institute for Agricultural Research, France.

6 KSU: Kansas State University, USA.

**Figure 1.** PCR amplification of the *Pm3b* allele by *Pm3b* specific molecular markers. The arrow indicates the expected band of size 1382bp which is diagnostic for *Pm3b*. The numbers 1 to 28 represent the tested accessions, where 2, 21, 22, 23, 24 and 26 possess *Pm3b*. M stands for 1kb marker ladder.



There are two earlier studies which determined the presence of *Pm3* alleles in some elite breeding lines as well as landraces [19,27]. From the *Pm3a-g* alleles, *Pm3b* and *Pm3c* were the only detected *Pm3* alleles in 30 and 4 landraces respectively, out of a total of 1320 landraces screened for the presence of *Pm3a* to *Pm3g* alleles by Kaur *et al.* [27]. *Pm3g* has been identified in one breeding line Oid HD4-219 which originated from France [19]. In addition, *Pm3d* has been detected in breeding line Oid HD4-266 (France) while *Pm3e* was found in a landrace from Tajikistan (TA10381) and a breeding line (Oid 91-35) from France [19]. These data support a recent evolution of at least some *Pm3* alleles in hexaploid wheat breeding material as *Pm3a*, *Pm3d*, *Pm3f* and *Pm3g* have not been detected in any of the wheat landraces screened to date [27], but only in advanced breeding material.

The data presented here provide a detailed overview on the presence of *Pm3* resistance alleles in the wheat germplasm. Similar studies on functional allelic diversity have also been made for *Vrn* locus responsible for vernalization requirements in wheat. A set of 56 spring wheat cultivars and breeding lines were assessed for the allelic composition of *Vrn-1* locus and it was found that the majority of the germplasm carried the dominant allele *Vrn-A1* alone or in combination with *Vrn-B1*, *Vrn-D1* or *Vrn-B3* alleles [33]. In another study, 278 Chinese wheat cultivars were characterized for the vernalization genes *Vrn-A1*, *-B1*, *-D1*, and *-B3*. The dominant *Vrn-D1* allele was detected with the highest frequency in the Chinese wheat cultivars (37.8%), followed by the dominant *Vrn-A1*, *-B1*, and *-B3* alleles [34].

### 3.2. The Susceptible *Pm3CS* Allele is Present in Accessions From Diverse Geographical Origins

Among the set of 109 accessions that possess the *Pm3* haplotype (see 3.1), the *Pm3a* to *Pm3g* alleles were detected in 28 accessions (*Pm3b*, *Pm3c*, *Pm3d*, *Pm3e* and *Pm3f*; see 3.1). The remaining 81 accessions from this set must either have different alleles of *Pm3* which could not be detected by the allele specific markers or they carry the widespread susceptible allele *Pm3CS*. *Pm3CS* is the consensus sequence of all *Pm3* alleles [18] and no *Pm3CS*-specific markers can be developed. Therefore, its presence can only be determined by amplification and sequencing of the complete gene from a particular accession. To test for the frequency of the *Pm3CS* allele in resistant germplasm having a *Pm3* haplotype, but none of the classical *Pm3a-g* alleles, we selected from the 81 accessions

described above a subset of 41 accessions and amplified the sequence of *Pm3* genes. The susceptible *Pm3* allele, *Pm3CS* was isolated from 8 different accessions originating from India (2), Australia (1), France (1), Canada (2), Ethiopia (1) and Tajikistan (1) (Table 2). Furthermore, out of the 41 accessions analysed, 15 accessions contained new *Pm3* sequences [35] and 18 accessions had the *Pm3Go/Jho* allele (see 3.3. below).

Similar observations have been made in previous studies, where *Pm3CS* was isolated from accessions of very different origins. *Pm3CS* was found to be the most frequently amplified sequence in a set of 45 resistant hexaploid wheat landraces, where it was identified in 9 accessions [9]. Yahiaoui *et al.* [19] also reported the isolation of *Pm3CS* from different breeding lines and cultivars (Table 2). *Pm3CS* has also been isolated from tetraploid wheat accessions, as reported in Yahiaoui *et al.* [20], indicating that *Pm3CS* is an ancient allele which was present in the wheat gene pool before wheat domestication and the evolution of hexaploid wheat. Therefore, *Pm3CS* has been proposed to be the ancestor of resistance alleles of *Pm3* [19].

These studies demonstrate that the susceptible ancestral sequence *Pm3CS* is present in accessions from many and very diverse geographical origins, both in the hexaploid as well as the tetraploid wheat gene pool. It is a very frequent allele of *Pm3* and has been identified in different types of wheat material, including landraces, breeding lines and cultivars (see Table 2).

### 3.3. Abundant Presence of the Transitional, Susceptible *Pm3Go/Jho* Allele in Accessions from Nepal, India and Bhutan

In addition to the amplification of *Pm3CS*, another previously reported susceptible allele, *Pm3Go/Jho*, was isolated from 18 accessions (among the 41 accessions subjected to *Pm3* amplification, see 3.2). We specifically identified this allele in accessions that originated from Nepal (13 accessions), India (4 accessions) and China (1 accession) (Table 2). This indicates a widespread occurrence of *Pm3Go/Jho* in Asia and specifically close to the Himalayan range. *Pm3Go/Jho* has been previously isolated from two bread wheat landraces PI481711Go and PI481723Jho, collected from high altitude (2800m) in Bhutan [19]. This allele was named *Pm3Go/Jho* after the name of accessions from which it was first isolated. *Pm3Go/Jho* encodes a protein with only one amino acid difference in comparison to *PM3CS* and this change at position 659 (W<sub>659</sub> instead of R<sub>659</sub>) is identical to the one in *PM3D* and *PM3E* proteins. *Pm3d* and *Pm3e* encode proteins that are highly similar to *PM3CS* and have only 3 and 2 amino acid differences to *PM3CS*, respectively. *Pm3Go/Jho* is a susceptible allele but the W<sub>659</sub> amino acid polymorphism is essential for *Pm3d* dependent resistance together with the other 2 polymorphic amino acids in *Pm3d* [19]. This indicates that *Pm3Go/Jho* represents a transitional allele, representing the evolutionary link between the ancestral sequence *Pm3CS* and the functional *Pm3d* and *Pm3e* alleles. This would suggest that the *Pm3d* and *Pm3e* alleles also originated in or near the Himalayan range.



**Table 2.** Summary of accessions and countries of origin from which the susceptible alleles *Pm3CS* and *Pm3Go/Jho* were isolated.

<i>Pm3</i> allele	Origin	Number of accessions	Accession (s)	Type	Source of accessions	Reference
<i>Pm3CS</i>	<b>Hexaploid wheat</b>					
	India	2	TRI2480, TRI2739	unknown	IPK <sup>1</sup>	This work
	Australia	1	TRI7243	unknown	IPK	This work
	France	1	TRI7345	unknown	IPK	This work
	Canada	2	TRI7736, TRI7741	unknown	IPK	This work
	Ethiopia	1	TRI15026	unknown	IPK	This work
	Tajikistan	1	TRI17510	unknown	IPK	This work
	Pakistan	2	AUS 4856, IG41554	Landrace	AWCC <sup>2</sup>	[9]
	Afghanistan	7	AWCC9947, AWCC14695, AWCC14849, AUS13655, AUS13656, AUS13704, AUS14526	Landrace	AWCC	[9]
	Turkey	2	IG42398, IG42869	Landrace	ICARDA <sup>3</sup>	[9]
	Iran	1	IG122584	Landrace	ICARDA	[9]
	China	1	Chinese Spring	Landrace	ART <sup>4</sup>	[19]
	Europe	5	Caribo, Greif, Obelisk, Kormoran, Monopol,	Cultivar	ART	[19]
	Belgium	1	Rouquin	Cultivar	ART	[19]
	Switzerland	1	Boval	Cultivar	ART	[19]
	Germany	1	Kanzler	Cultivar	ART	[19]
	France	1	Oid HD4-234	Breeding line	INRA <sup>5</sup>	[19]
	UK	1	Maris Huntsman	Cultivar	ART	[19]
	USA	1	Thatcher	Cultivar	ART	[19]
	Tajikistan	1	TA 10384	Landrace	KSU <sup>6</sup>	[19]
<i>Pm3CS</i>	<b>Tetraploid wheat</b>					
	Turkey	5	PI560872, PI560874, PI428145, PI428053, IG116184	<i>T. dicoccoides</i>	USDA/ARS <sup>7</sup> / ICARDA	[20]
	Ethiopia	1	PI58789	<i>T. dicoccum</i>	USDA/ARS	[20]
<i>Pm3Go/Jho</i>	Ethiopia	1	CItr14846	<i>T. durum</i>	USDA/ARS	[20]
	<b>Hexaploid wheat</b>					
	India	4	TRI2596, TRI3197, TRI3535, TRI3992	unknown	IPK	This work
	Nepal	13	TRI2611, TRI2889, TRI3232, TRI3628, TRI4359, TRI11131, TRI11132, TRI11133, TRI11135, TRI11136, TRI11137, TRI11139, TRI11151	unknown	IPK	This work
	China	1	TRI14752	unknown	IPK	This work

1 IPK: Leibniz Institute of Plant Genetics and Crop Plant Research, Germany.

2 AWCC: Australian Winter Cereals Collection, Australia.

3 ICARDA: International Centre for Agricultural Research in the Dry Areas, Syria.

4 ART: Agroscope Reckenholz-Tänikon Research Station, Switzerland.

5 INRA: French National Institute for Agricultural Research, France.

6 KSU: Kansas State University, USA.

7 USDA/ARS: United States Department of Agriculture/Agricultural Research Service, USA.

### 3.4. Widespread Existence of the *Pm3b* Resistance Allele in Landraces from Afghanistan

The second set of genetic material analyzed in this study consisted of 272 landraces from Afghanistan. We phenotyped these accessions for powdery mildew resistance by infecting them with four different isolates. Thirty-nine out of 272 accessions were found to be resistant or intermediately resistant to at least one of the isolate tested (Figure 2a, 2c; Appendix 1). These 272 accessions were also screened for the *Pm3* haplotype using a specific STS marker (see above). The *Pm3* haplotype was present at a high frequency in 236 accessions out of 272 (86.7%) (Appendix 1, Figure 2a, 2b). These 236 accessions were then screened for the presence of known *Pm3* alleles (*Pm3a*-*Pm3g*) using *Pm3* allele specific markers. The *Pm3b* allele was found to be the only known functional *Pm3* allele present in this subset and was detected in twelve landraces (Figure 1, Appendix 1). The *Pm3b* allele was found to be well distributed geographically in the wheat growing regions of Afghanistan and was detected in accessions that originated from Herat, Badghis, Vardak, Parvan and Ghazni provinces (Figure 2a). Among the 39 powdery mildew resistant accessions (Figure 2a and 2c), 12 landraces had the *Pm3b* allele (32.4%) (Figure 2a) and two did not possess the *Pm3* haplotype (Figure 2c). These data suggest that the *Pm3b* allele is possibly the only active *Pm3* resistance gene in Afghanistan landraces. We conclude that *Pm3b* is a very frequent source of the observed resistance in landraces in Afghanistan and the resistance in the remaining 27 landraces with a resistance phenotype must be caused either by genes different from *Pm3* alleles, by the recently characterized *Pm3k-r* alleles, or by new, unknown *Pm3* alleles. In a previous study, large sets of landraces originating from Turkey (420 landraces), Iran (393 landraces) and Pakistan (131 landraces) were screened for the presence of the *Pm3b* alleles and it was only found in 1, 6 and 0 landraces from each of these country sets, respectively (Table 1) [9, 27].

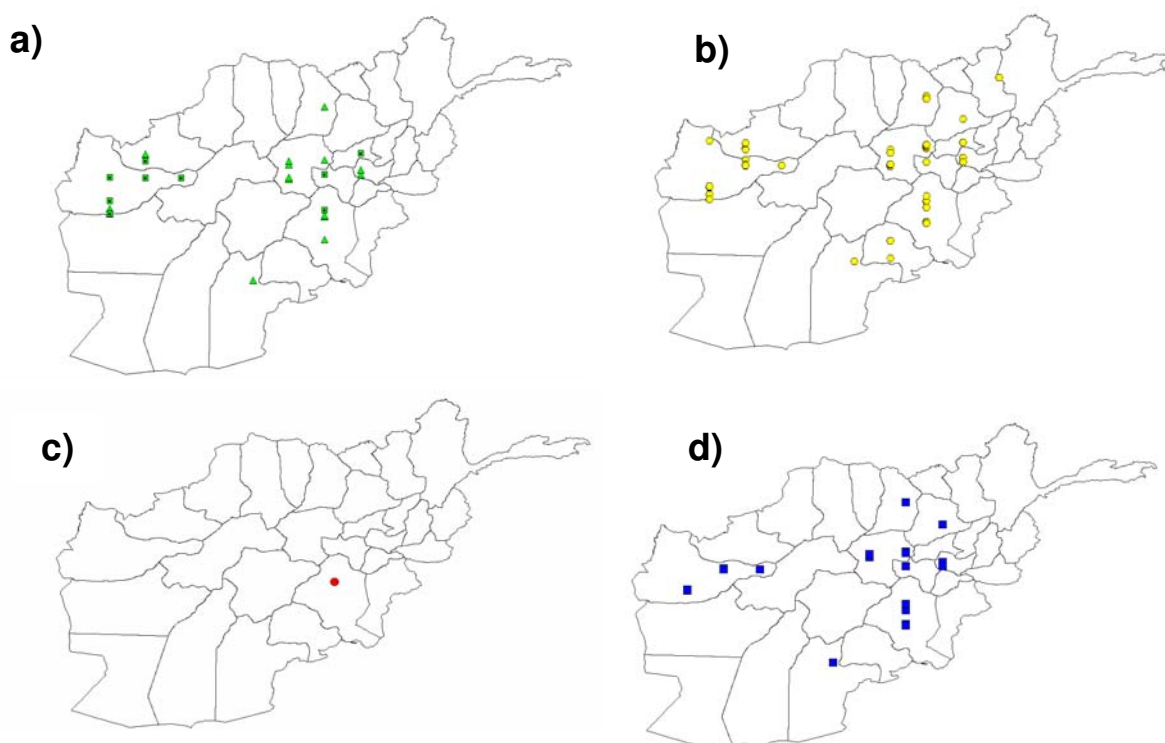
The high percentage of 236 accessions being susceptible (84.3%) but having the *Pm3* haplotype (Figure 2b) suggest that the *Pm3* alleles in these lines do not correspond to *Pm3* resistance alleles and that susceptible alleles such as *Pm3CS* or *Pm3Go/Jho* must be widespread among the landraces. Evidently, the high frequency of the *Pm3* haplotype provides an ideal genetic background for the mutational development of active resistance genes with new specificities in recent evolutionary times. The accessions with susceptible and resistance alleles of *Pm3* originated in geographical vicinity of each other in Afghanistan (Figure 2a, 2b). It was not possible to define particular geographic areas for *Pm3* resistance and susceptible alleles (Figure 2a, 2b).

## 4. Conclusions

In this work, we have studied the *Pm3* allele distribution in a diverse set of more than 1000 accessions from wheat gene banks. We found a widespread occurrence of susceptible *Pm3* alleles. The *Pm3CS* sequence was present globally and a second susceptible allele, *Pm3Go/Jho* was frequent in accessions from the Himalayan region. Interestingly, *Pm3Go/Jho* allele is a transitional allele between *Pm3CS* and the resistant *Pm3d* allele, which was originally described in an accession from Afghanistan (Hindukush). Therefore, it is likely that *Pm3d* is a derivative by mutation of *Pm3Go/Jho* and originated somewhere in the geographical region where *Pm3Go/Jho* is frequent. Interestingly, the *Pm3e* allele, although first described in an Australian accession, was also found in the geographical area of *Pm3Go/Jho* i.e., in two accessions from India. As the PM3E protein differs only by one amino acid from PM3Go/Jho, we propose an origin in the Himalayan region also for *Pm3e* allele. From the

seven functional *Pm3* resistance alleles *Pm3a-g*, only *Pm3b* and *Pm3c* were frequently identified in the gene bank material analysed. Our data provide good evidence that *Pm3b* originates from a geographical region centered around Afghanistan, whereas *Pm3c* is frequently found in accessions from Nepal and India and possibly evolved there. Given these data on the functional alleles, it is likely that the region of the Himalaya and surrounding geographical areas have been a hotspot for the evolution of new *Pm3* resistance alleles. This suggests that the search for new functional *Pm3* alleles should be focused on genetic material from these regions.

**Figure 2.** Geographical origin of the entire set of 272 landraces from Afghanistan. Several accessions originated from identical geographic sites and therefore, the number of geographical identifiers is not identical to the number of accessions. (a) Accessions that carry the *Pm3* haplotype and are resistant to at least one of the isolates tested are indicated. The green squares with a dot indicate the presence of *Pm3b* resistance allele and the green triangles indicate the accessions that possess the *Pm3* haplotype but none of the known alleles *Pm3a* to *Pm3g*. (b) Yellow circles indicate the accessions that carry the *Pm3* haplotype but are susceptible to the powdery mildew isolates tested. (c) The red circle marks the origin of two accessions that do not have a *Pm3* haplotype but are resistant to at least one of the isolates tested. (d) Blue squares mark the accessions that do not have the *Pm3* haplotype and are susceptible to powdery mildew isolates tested.



## Acknowledgements

We thank the gene banks of IPK, Gatersleben, Germany and AWCC, Australia for providing the wheat accessions and the bread wheat landraces from Afghanistan used in this study. We thank

Susanne Brunner and Nabila Yahiaoui for helpful discussion and comments. This study was supported by the Swiss National Science Foundation grant 31003A-127061/1 to BK.

## References

- 1 Tanksley, S.D.; McCouch, S.R. Seed banks and molecular maps: Unlocking genetic potential from the wild. *Science* **1997**, *277*, 1063-1066.
- 2 Hoisington, D.; Khairallah, M.; Reeves, T.; Ribaut, J.; Skovmand, B.; Taba, S.; Warburton, M. Plant genetic resources: What can they contribute toward increased crop productivity? *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5937-5943.
- 3 Huang, X.Q.; Börner, A.; Börner, M.S.; Ganai, M.W. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theor. Appl. Genet.* **2002**, *105*, 699-707.
- 4 Mondini, L.; Noorani, A.; Pagnotta, M.A. Assessing plant genetic diversity by molecular tools. *Diversity* **2009**, *1*, 19-35.
- 5 Warburton, M.L.; Crossa, J.; Franco, J.; Kazi, M.; Trethowan, R.; Rajaram, S.; Pfeiffer, W.; Zhang, P.; Dreisigacker, S.; van Ginkel, M. Bringing wild relatives back into the family: Recovering genetic diversity of CIMMYT bread wheat germplasm. *Euphytica*. **2006**, *149*, 289-301.
- 6 Roussel, V.; Koenig, J.; Beckert, M.; Balfourier, F. Molecular diversity in french bread wheat accessions related to temporal trends and breeding programmes. *Theor. Appl. Genet.* **2004**, *108*, 920-930.
- 7 Hao, C.Y.; Zhang, X.Y.; Wang, L.F.; Dong, Y.S.; Shang, X.W.; Jia, J.Z. Genetic diversity and core collection evaluations in common wheat germplasm from the Northwestern Spring Wheat Region in China. *Mol. Breed.* **2006**, *17*, 69-77.
- 8 Landjeva, S.; Korzun, V.; Börner, A. Molecular markers: actual and potential contributions to wheat genome characterization and breeding. *Euphytica* **2007**, *156*, 271-296.
- 9 Bhullar, N.K.; Street, K.; Mackay, M.; Yahiaoui, N.; Keller, B. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 9519-9524.
- 10 Uauy, C.; Distelfeld, A.; Fahima, T.; Blechl, A.; Dubcovsky, J.A. NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **2006**, *314*, 1298-1301.
- 11 Yan, L.; Loukoianov, A.; Tranquilli, G.; Helguera, M.; Fahima, T.; Dubcovsky, J. Positional cloning of the wheat vernalization gene *Vrn1*. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6263-6268.
- 12 Yan, L.; Loukoianov, A.; Blechl, A.; Tranquilli, G.; Ramakrishna, W.; SanMiguel, P.; Bennetzen, J.L.; Echenique, V.; Dubcovsky, J. The wheat *Vrn2* gene is a flowering repressor down-regulated by vernalization. *Science* **2004**, *303*, 1640-1644.
- 13 Faris, J.D.; Fellers, J.P.; Brooks, S.A.; Gill, B.S. A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of a candidate gene. *Genetics* **2003**, *164*, 311-321.

- 14 Huang, L.; Brooks, S.A.; Li, W.; Fellers, J.P.; Trick, H.N.; Gill, B.S. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics* **2003**, *164*, 655-664.
- 15 Feuillet, C.; Travella, S.; Stein, N.; Albar, L.; Nublat, A.; Keller, B. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15253-15258.
- 16 Cloutier, S.; McCallum, B.D.; Loutre, C.; Banks, T.W.; Wicker, T.; Feuillet, C.; Keller, B.; Jordan, M.C. Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. *Plant Mol. Biol.* **2007**, *65*, 93-106.
- 17 Krattinger, S.G.; Lagudah, E.S.; Spielmeyer, W.; Singh, R.P.; Huerta-Espino, J.; McFadden, H.; Bossolini, E.; Selter, L.L.; Keller, B. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* **2009**, *323*, 1360-1363.
- 18 Yahiaoui, N.; Srichumpa, P.; Dudler, R.; Keller, B. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. *Plant J.* **2004**, *37*, 528-538.
- 19 Yahiaoui, N.; Brunner, S.; Keller, B. Rapid generation of new powdery mildew resistance genes after wheat domestication. *Plant J.* **2006**, *47*, 85-98.
- 20 Yahiaoui, N.; Kaur, N.; Keller, B. Independent evolution of functional *Pm3* resistance genes in wild tetraploid wheat and domesticated bread wheat. *Plant J.* **2009**, *57*, 846-856.
- 21 Srichumpa, P.; Brunner, S.; Keller, B.; Yahiaoui, N. Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexaploid bread wheat. *Plant Physiol.* **2005**, *139*, 885-895.
- 22 Rong, J.K.; Millet, E.; Manisterski, J.; Feldman, M. A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP-based mapping. *Euphytica* **2000**, *115*, 121-126.
- 23 Blanco, A.; Gadaleta, A.; Cenci, A.; Carluccio, A.V.; Abdelbacki, A.M.; Simeone, R. Molecular mapping of the novel powdery mildew resistance gene *Pm36* introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat. *Theor. Appl. Genet.* **2008**, *117*, 135-142.
- 24 Perugini, L.D.; Murphy, J.P.; Marshal, D.; Brown-Guedira, G. *Pm37*, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*. *Theor. Appl. Genet.* **2008**, *116*, 417-425.
- 25 Bougot, Y.; Lemoine, J.; Pavoine, M.T.; Barloy, D.; Doussinault G. Identification of a microsatellite marker associated with *Pm3* resistance alleles to powdery mildew in wheat. *Plant Breeding* **2002**, *121*, 325-329.
- 26 Tommasini, L.; Yahiaoui, N.; Srichumpa, P.; Keller, B. Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. *Theor. Appl. Genet.* **2006**, *114*, 165-175.
- 27 Kaur, N.; Street, K.; Mackay, M.; Yahiaoui, N.; Keller, B. Molecular approaches for characterization and use of natural disease resistance in wheat. *Eur. J. Plant Pathol.* **2008**, *121*, 387-397.
- 28 Schweizer, P.; Christoffel, A.; Dudler, R. Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance. *Plant J.* **1999**, *20*, 541-552.

- 29 Briggles, L.W. Three Loci in wheat involving resistance to *Erysiphe graminis* f. sp. *tritici*. *Crop Sci.* **1966**, *6*, 461-465.
- 30 Bennett, F.G.A. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. *Plant Pathol.* **1984**, *33*, 279-300.
- 31 Hsam, S.L.K.; Zeller, F.J. Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In *The Powdery Mildews: A Comprehensive Treatise*; Be'langier, R.R., Bushnell, W.R., Dik, A.J., Carver, T.L.W., Eds.; APS Press: St Paul, MN, USA, 2002; pp. 219-238.
- 32 Zeller, F.J.; Lutz, J.; Stephan, U. Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.) 1. *Mlk* and other alleles at the *Pm3* locus. *Euphytica* **1993**, *68*, 223-229.
- 33 Santra, D.K.; Santra, M.; Allan, R.E.; Campbell, K.G.; Kidwell, K.K. Genetic and molecular characterization of vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* in spring wheat germplasm from the pacific northwest region of the USA. *Plant breeding* **2009**, *128*, 576 -584.
- 34 Zhang, X.K.; Xiao, Y.G.; Zhang, Y.; Xia, X.C.; Dubcovsky, J.; He, Z.H. Allelic variation at the vernalization genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* in chinese wheat cultivars and their association with growth habit. *Crop Sci.* **2008**, *48*, 458-470.
- 35 Bhullar, N.K.; Zhang, Z.; Wicker, T.; Keller, B. Wheat gene bank accessions as a source of new alleles of the powdery mildew resistance gene *Pm3*: a large scale allele mining project. *BMC Plant Biol.* **2010**, *10*, 88.

**Appendix 1.** Summary of the *Pm3* characterization in the set of 272 landraces originating from Afghanistan. The symbol P refers to the presence of the *Pm3* haplotype, while A refers to absence of the *Pm3* haplotype. From the alleles *Pm3a-g*, only *Pm3b* was detected in 12 accessions among this set. Thirty-nine accessions that were found resistant or intermediately resistant to at least one of the tested isolates are marked in bold. Thirty-seven of these 39 accessions possess the *Pm3* haplotype.

	CODE	Accession Number	Origin	<i>Pm3</i> haplotype	<i>Pm3</i> allele detected when tested with allele-specific primers for <i>Pm3a</i> to <i>Pm3g</i>
1	<b>AUS</b>	<b>9939</b>	Afghanistan	P	-
2	AUS	9940	Afghanistan	P	-
3	AUS	9941	Afghanistan	P	-
4	<b>AUS</b>	<b>9943</b>	Afghanistan	P	<i>Pm3b</i>
5	AUS	9944	Afghanistan	P	-
6	AUS	9945	Afghanistan	A	-
7	AUS	9947	Afghanistan	P	-
8	<b>AUS</b>	<b>9948</b>	Afghanistan	P	<i>Pm3b</i>
9	AUS	9949	Afghanistan	P	-
10	AUS	9950	Afghanistan	P	-
11	AUS	9951	Afghanistan	A	-
12	AUS	9952	Afghanistan	A	-
13	AUS	9963	Afghanistan	P	-
14	AUS	9964	Afghanistan	P	-

## Appendix 1. Cont.

15	AUS	9965	Afghanistan	P	-
16	AUS	9966	Afghanistan	P	-
17	AUS	9997	Afghanistan	P	-
18	AUS	9998	Afghanistan	P	-
19	AUS	9999	Afghanistan	P	-
20	AUS	10000	Afghanistan	P	-
21	AUS	10001	Afghanistan	P	-
22	AUS	10002	Afghanistan	P	-
23	<b>AUS</b>	<b>10003</b>	Afghanistan	P	<i>Pm3b</i>
24	AUS	10027	Afghanistan	P	-
25	AUS	10028	Afghanistan	A	-
26	AUS	10029	Afghanistan	P	-
27	AUS	10030	Afghanistan	P	-
28	AUS	10031	Afghanistan	P	-
29	AUS	10032	Afghanistan	P	-
30	<b>AUS</b>	<b>10033</b>	Afghanistan	P	<i>Pm3b</i>
31	AUS	10034	Afghanistan	A	-
32	AUS	10035	Afghanistan	P	-
33	AUS	10036	Afghanistan	P	-
34	<b>AUS</b>	<b>10963</b>	Afghanistan	P	-
35	<b>AUS</b>	<b>13239</b>	Afghanistan	P	<i>Pm3b</i>
36	AUS	13240	Afghanistan	P	-
37	AUS	13241	Afghanistan	P	-
38	AUS	13274	Afghanistan	P	-
39	AUS	13275	Afghanistan	P	-
40	<b>AUS</b>	<b>13276</b>	Afghanistan	P	-
41	AUS	13277	Afghanistan	P	-
42	AUS	13285	Afghanistan	A	-
43	AUS	13290	Afghanistan	P	-
44	AUS	13291	Afghanistan	P	-
45	<b>AUS</b>	<b>13292</b>	Afghanistan	P	-
46	AUS	13293	Afghanistan	P	-
47	AUS	13294	Afghanistan	P	-
48	AUS	13295	Afghanistan	P	-
49	AUS	13296	Afghanistan	P	-
50	<b>AUS</b>	<b>13297</b>	Afghanistan	P	<i>Pm3b</i>
51	AUS	13298	Afghanistan	P	-
52	AUS	13299	Afghanistan	P	-
53	<b>AUS</b>	<b>13300</b>	Afghanistan	P	-
54	AUS	13301	Afghanistan	A	-
55	AUS	13302	Afghanistan	P	-
56	AUS	13303	Afghanistan	P	-
57	AUS	13304	Afghanistan	P	-
58	AUS	13305	Afghanistan	P	-

## Appendix 1. Cont.

59	AUS	13306	Afghanistan	P	<i>Pm3b</i>
60	AUS	13307	Afghanistan	P	<i>Pm3b</i>
61	AUS	13309	Afghanistan	P	-
62	AUS	13310	Afghanistan	A	-
63	AUS	13311	Afghanistan	P	<i>Pm3b</i>
64	AUS	13312	Afghanistan	A	-
65	AUS	13313	Afghanistan	A	-
66	AUS	13314	Afghanistan	P	-
67	AUS	13315	Afghanistan	A	-
68	AUS	13636	Afghanistan	P	-
69	AUS	13637	Afghanistan	P	-
70	AUS	13638	Afghanistan	P	-
71	AUS	13639	Afghanistan	P	-
72	AUS	13640	Afghanistan	P	-
73	AUS	13641	Afghanistan	P	-
74	AUS	13642	Afghanistan	P	-
75	AUS	13643	Afghanistan	P	-
76	AUS	13644	Afghanistan	P	-
77	AUS	13645	Afghanistan	P	-
78	AUS	13646	Afghanistan	P	-
79	AUS	13647	Afghanistan	P	-
80	AUS	13654	Afghanistan	P	-
81	AUS	13655	Afghanistan	P	-
82	AUS	13656	Afghanistan	P	-
83	AUS	13657	Afghanistan	P	-
84	AUS	13658	Afghanistan	P	-
85	AUS	13659	Afghanistan	P	-
86	AUS	13660	Afghanistan	P	-
87	AUS	13661	Afghanistan	P	-
88	AUS	13662	Afghanistan	P	-
89	AUS	13663	Afghanistan	P	-
90	AUS	13664	Afghanistan	P	-
91	AUS	13665	Afghanistan	P	-
92	AUS	13666	Afghanistan	P	-
93	AUS	13703	Afghanistan	P	-
94	AUS	13704	Afghanistan	P	-
95	AUS	13705	Afghanistan	P	-
96	AUS	13706	Afghanistan	P	-
97	AUS	13707	Afghanistan	P	-
98	AUS	13708	Afghanistan	A	-
99	AUS	13723	Afghanistan	A	-
100	AUS	13724	Afghanistan	P	-
101	AUS	13725	Afghanistan	P	-
102	AUS	13726	Afghanistan	P	-



## Appendix 1. Cont.

103	AUS	13727	Afghanistan	P	-
104	AUS	13728	Afghanistan	P	-
105	AUS	13729	Afghanistan	P	-
106	AUS	13730	Afghanistan	P	-
107	AUS	13731	Afghanistan	P	-
108	AUS	13732	Afghanistan	P	-
109	AUS	13733	Afghanistan	P	-
110	AUS	13734	Afghanistan	A	-
111	AUS	13735	Afghanistan	P	-
112	AUS	13736	Afghanistan	A	-
113	AUS	13737	Afghanistan	P	-
114	AUS	13738	Afghanistan	P	-
115	<b>AUS</b>	<b>14442</b>	Afghanistan	P	-
116	AUS	14443	Afghanistan	P	-
117	<b>AUS</b>	<b>14444</b>	Afghanistan	P	-
118	AUS	14446	Afghanistan	P	-
119	AUS	14447	Afghanistan	P	-
120	AUS	14448	Afghanistan	P	-
121	<b>AUS</b>	<b>14449</b>	Afghanistan	P	-
122	AUS	14450	Afghanistan	A	-
123	AUS	14451	Afghanistan	P	-
124	AUS	14452	Afghanistan	P	-
125	AUS	14454	Afghanistan	P	-
126	AUS	14455	Afghanistan	P	-
127	AUS	14456	Afghanistan	P	-
128	AUS	14457	Afghanistan	P	-
129	AUS	14458	Afghanistan	P	-
130	AUS	14459	Afghanistan	P	-
131	AUS	14461	Afghanistan	P	-
132	AUS	14463	Afghanistan	P	-
133	AUS	14474	Afghanistan	P	-
134	<b>AUS</b>	<b>14475</b>	Afghanistan	P	-
135	AUS	14476	Afghanistan	P	-
136	AUS	14480	Afghanistan	P	-
137	AUS	14481	Afghanistan	P	-
138	AUS	14482	Afghanistan	P	-
139	AUS	14483	Afghanistan	P	-
140	AUS	14484	Afghanistan	P	-
141	AUS	14485	Afghanistan	P	-
142	AUS	14486	Afghanistan	P	-
143	AUS	14487	Afghanistan	P	-
144	AUS	14488	Afghanistan	P	-
145	AUS	14489	Afghanistan	P	-
146	AUS	14490	Afghanistan	P	-

## Appendix 1. Cont.

147	AUS	14491	Afghanistan	P	-
148	AUS	14492	Afghanistan	P	-
149	AUS	14493	Afghanistan	P	-
150	AUS	14494	Afghanistan	P	-
151	AUS	14495	Afghanistan	P	-
152	AUS	14496	Afghanistan	P	-
153	AUS	14497	Afghanistan	P	-
154	AUS	14498	Afghanistan	P	-
155	AUS	14499	Afghanistan	P	-
156	AUS	14501	Afghanistan	P	-
157	AUS	14502	Afghanistan	P	-
158	AUS	14503	Afghanistan	P	-
159	<b>AUS</b>	<b>14504</b>	Afghanistan	P	<i>Pm3b</i>
160	AUS	14505	Afghanistan	P	-
161	AUS	14506	Afghanistan	P	-
162	AUS	14513	Afghanistan	P	-
163	AUS	14514	Afghanistan	P	-
164	AUS	14515	Afghanistan	P	-
165	AUS	14516	Afghanistan	P	-
166	<b>AUS</b>	<b>14517</b>	Afghanistan	P	-
167	AUS	14518	Afghanistan	P	-
168	AUS	14519	Afghanistan	P	-
169	AUS	14520	Afghanistan	P	-
170	AUS	14521	Afghanistan	P	-
171	AUS	14522	Afghanistan	P	-
172	AUS	14523	Afghanistan	P	-
173	AUS	14524	Afghanistan	P	-
174	AUS	14525	Afghanistan	P	-
175	<b>AUS</b>	<b>14526</b>	Afghanistan	P	-
176	AUS	14527	Afghanistan	P	-
177	AUS	14528	Afghanistan	P	-
178	AUS	14531	Afghanistan	P	-
179	<b>AUS</b>	<b>14532</b>	Afghanistan	P	<i>Pm3b</i>
180	AUS	14535	Afghanistan	P	-
181	AUS	14546	Afghanistan	A	-
182	AUS	14547	Afghanistan	P	-
183	AUS	14565	Afghanistan	P	-
184	AUS	14566	Afghanistan	P	-
185	AUS	14567	Afghanistan	P	-
186	AUS	14568	Afghanistan	P	-
187	AUS	14569	Afghanistan	P	-
188	AUS	14605	Afghanistan	A	-
189	AUS	14606	Afghanistan	P	-
190	AUS	14607	Afghanistan	P	-

## Appendix 1. Cont.

191	AUS	14608	Afghanistan	P	-
192	<b>AUS</b>	<b>14609</b>	Afghanistan	P	-
193	AUS	14610	Afghanistan	P	-
194	AUS	14611	Afghanistan	P	-
195	AUS	14612	Afghanistan	P	-
196	AUS	14613	Afghanistan	P	-
197	AUS	14614	Afghanistan	P	-
198	AUS	14624	Afghanistan	P	-
199	<b>AUS</b>	<b>14625</b>	Afghanistan	P	-
200	<b>AUS</b>	<b>14626</b>	Afghanistan	P	-
201	AUS	14627	Afghanistan	P	-
202	AUS	14628	Afghanistan	P	-
203	AUS	14629	Afghanistan	A	-
204	AUS	14630	Afghanistan	A	-
205	AUS	14631	Afghanistan	P	-
206	AUS	14632	Afghanistan	P	-
207	AUS	14633	Afghanistan	A	-
208	AUS	14634	Afghanistan	P	-
209	AUS	14635	Afghanistan	A	-
210	AUS	14636	Afghanistan	P	-
211	AUS	14637	Afghanistan	P	-
212	AUS	14638	Afghanistan	P	-
213	AUS	14639	Afghanistan	P	-
214	AUS	14640	Afghanistan	P	-
215	AUS	14641	Afghanistan	P	-
216	AUS	14642	Afghanistan	A	-
217	AUS	14643	Afghanistan	P	-
218	AUS	14644	Afghanistan	P	-
219	AUS	14645	Afghanistan	P	-
220	AUS	14646	Afghanistan	P	-
221	AUS	14647	Afghanistan	P	-
222	AUS	14648	Afghanistan	P	-
223	AUS	14649	Afghanistan	P	-
224	AUS	14650	Afghanistan	P	-
225	AUS	14689	Afghanistan	A	-
226	<b>AUS</b>	<b>14690</b>	Afghanistan	P	-
227	<b>AUS</b>	<b>14691</b>	Afghanistan	P	-
228	AUS	14692	Afghanistan	P	-
229	AUS	14693	Afghanistan	P	-
230	AUS	14694	Afghanistan	P	-
231	AUS	14695	Afghanistan	P	-
232	AUS	14696	Afghanistan	P	-
233	AUS	14697	Afghanistan	P	-
234	AUS	14698	Afghanistan	P	-

## Appendix 1. Cont.

235	AUS	14699	Afghanistan	A	-
236	AUS	14700	Afghanistan	P	-
237	AUS	14701	Afghanistan	P	-
238	AUS	14702	Afghanistan	P	-
239	<b>AUS</b>	<b>14703</b>	Afghanistan	P	-
240	<b>AUS</b>	<b>14704</b>	Afghanistan	A	-
241	<b>AUS</b>	<b>14705</b>	Afghanistan	P	-
242	<b>AUS</b>	<b>14706</b>	Afghanistan	A	-
243	AUS	14707	Afghanistan	P	-
244	AUS	14708	Afghanistan	P	-
245	AUS	14709	Afghanistan	P	-
246	AUS	14710	Afghanistan	P	-
247	AUS	14711	Afghanistan	A	-
248	AUS	14713	Afghanistan	P	-
249	AUS	14714	Afghanistan	P	-
250	AUS	14715	Afghanistan	P	-
251	<b>AUS</b>	<b>14840</b>	Afghanistan	P	<i>Pm3b</i>
252	AUS	14841	Afghanistan	P	-
253	AUS	14842	Afghanistan	P	-
254	AUS	14843	Afghanistan	A	-
255	AUS	14844	Afghanistan	A	-
256	AUS	14845	Afghanistan	P	-
257	AUS	14846	Afghanistan	P	-
258	AUS	14847	Afghanistan	P	-
259	AUS	14848	Afghanistan	P	-
260	<b>AUS</b>	<b>14849</b>	Afghanistan	P	-
261	AUS	14850	Afghanistan	A	-
262	AUS	14851	Afghanistan	A	-
263	AUS	14852	Afghanistan	P	-
264	AUS	15209	Afghanistan	A	-
265	AUS	15210	Afghanistan	A	-
266	AUS	15212	Afghanistan	P	-
267	AUS	15218	Afghanistan	P	-
268	AUS	15320	Afghanistan	A	-
269	AUS	15321	Afghanistan	P	-
270	AUS	15624	Afghanistan	P	-
271	AUS	17502	Afghanistan	P	-
272	AUS	17503	Afghanistan	A	-